The foliage terpenoids of *Callitropsis nootkatensis*: Comparison of oils from cultivated and natural trees

Robert P. Adams
Biology Department, Baylor University, Box 97388, Waco, TX 76798 USA

John H. Russell
British Columbia Ministry of Forests, Lands and Natural Resource Operations, Cowichan Lake Research Station, Box 335, 7060 Forestry Road, Mesachie Lake, B. C. V0R 2N0 Canada

Gay Hunter
Museum Curator, Olympic National Park, 600 East Park Ave., Port Angeles, WA 98362

Thomas A. Fairhall
1136 Water St., Suite 101, Port Townsend, WA 98368

Karen Dillman
USFS, Tongass National Forest, P. O. Box 309, Petersburg, AK 9983

and

Mark Corbet
7376 Southwest McVey Ave., Redmond, OR 97756

ABSTRACT

Variation in the leaf terpenoids of *Callitropsis nootkatensis* was examined between cultivated and natural trees for four cases: **Case I.** Comparison of individual terpene concentrations and oil yields of Rennell Sound Pass trees grown in two sites (CLRS, JR) on Vancouver Island, BC revealed two significant (myrcene, α-terpinyl acetate) and one highly significant difference (terpinolene). No significant difference was found in % oil yield. PCO ordination showed that the CLRS and JR samples were interspersed, not separated by plantation site, suggesting the variation was mostly under genetic control rather than environmentally induced. **Case II.** ANOVA of oils from cultivated trees (CLRS site) compared to natural trees at John Day, OR showed that the concentrations of five of the six major monoterpenes were significantly or highly significantly different. However, % oil yield was not significantly different. PCO ordination revealed 3 chemotypes in which natural (John Day) trees were interspersed, implying that genetically controlled differences may be largely responsible for the ordination pattern. **Case III.** ANOVA of oils from cultivated trees at JR site compared to natural trees at Mt. Angeles, WA revealed two terpenes were highly significant different and another significant different in concentration. There was no significant difference in % oil yields. PCO showed the samples from cultivated and natural trees were mostly interspersed, pointing to genetic control rather than environmental factors for the pattern. **Case IV.** ANOVA comparing oils from cultivated trees (CLRS) verses natural trees at Mitkof Island, AK found no significant differences in any terpenoid or % oil yields. However, two chemotypes were found among the terpenoids in both cultivated and natural trees. The Mitkof Island case presents clear evidence that environmentally induced differences between oils from cultivated or natural trees has far less of an influence in comparison to chemotype (genetic) differences among individuals whether from cultivation, or natural populations of *C. nootkatensis*. Overall, in these four cases, it appears that the genetic component is much greater than the environmental component in determining both oil yields, and composition in the *C. nootkatensis* studied.
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KEY WORDS: *Callitropsis nootkatensis* (*Chamaecyparis nootkatensis*), terpenes, environment, Alaska yellow cedar.

The most complete, early analysis of the leaf oil of *Chamaecyparis nootkatensis* (treated as *Callitropsis nootkatensis* in this paper) was by Cheng and von Rudloff (1970). Subsequently, trans-totarol was isolated from *Chamaecyparis nootkatensis* (Constantine et al., 2001). Cool (2001) found new ent-daucane and acoranes from *xCupressocyparis leylandii*, but the compounds were not found in either of the putative parents (*Chamaecyparis nootkatensis*, *Cupressus macrocarpa*). Von Rudloff (1975) and Yatagai et al. (1985) published additional leaf oil compositional data based on one or a few individual trees. The most recent complete analysis of the volatile leaf oil of *Chamaecyparis nootkatensis* (as *Xanthocyparis nootkatensis*) is by Adams et al. 2007.

There does not appear to be a study of the genetic verses environment effects on the oil yields and terpenoid composition of *C. nootkatensis*. To examine these effects, four cases were chosen for this study. Case I. Trees grown at two sites on southern Vancouver Island (CLRS, Cowichan Lake Research Station, planted in 1988, 24 yr old and JR, Jordan River, planted in 1994, 18 yr old) were available for study and represented an unusual opportunity to examine the leaf oils from the same population growing in two different plots. Case II. Comparison of oils from natural trees at the Cedar Grove Botanical Area (near John Day, OR) compared to cultivated trees at CLRS (from cuttings taken at Cedar Grove). Case III. Comparison of oils from natural trees at Mt. Angeles, Olympic National Park, WA (ONP) compared to cultivated trees at JR. Case IV. Comparison of oils from natural trees at Mitkof Island, AK compared to cultivated trees at CLRS.

MATERIALS AND METHODS

Callitropsis nootkatensis plant materials - (samples taken from CLRS or JR sites, Nov. 2012):

Rennell Sound Pass, at JR, Lab Acc. *Adams 13701-13705*: JR 225, 231, 233, 234, Rennell Sound Pass; Haida Gwaii, BC, 53° 22' N, 132° 18' W, 280m;

Rennell Sound Pass, at CLRS, Lab Acc. *Adams 13706-13709*: CLRS 563, 564, 567, 569, 570, Rennell Sound Pass; Haida Gwaii, BC, 53° 22' N, 132° 18' W, 280m;

John Day, cultivated at CLRS, Lab Acc. *Adams 13664-13667*, CLRS 343, 345, 346, 347, Cedar Grove Botanical Area (CGCA), John Day, OR, 44° 21' N, 119° 20' W, ca. 1730m;

John Day, natural trees , Cedar Grove Botanical Area (CGCA), near John Day, OR, *Adams 14112-14117, 14121-14122,* ex *Mark Corbet #1-8,* 6 Oct 2013, 44° 20' 15.6" N; 119° 20' 17.1"W. elev. 1730 m, Malheur Co., OR.

Mt. Angeles (lower slope), Olympic National Park (ONP), cultivated at JR site, Lab Acc. *Adams 13673-13677*, JR 312, 313, 314, 316, 317, Mt. Angeles, WA, 47° 59' N, 123° 28'W, 1405 m;

Mt. Angeles (lower slope), Olympic National Park (ONP), natural trees, *Hunter and Fairhall 1-6*, 20 Oct. 2013, Lab Acc. *Adams 14138 - 14143,* Mt. Angeles, ONP, WA, 47° 58.96' N, 123° 27.99' W, elev. 1370 m.;

Mitkof Island, AK, cultivated at CLRS site, Lab Acc. *Adams 13710-13714*: CLRS 374, 375, 377, 380, 381, Mitkof Isl, AK, 56° 49' N, 132° 57' W, ca. 30m;

Mitkof Island, AK, natural trees, *K. Dillman, 1-5*, 23 Oct. 2013, Lab Acc. *Adams 14149-14153*, Mitkof Island, AK, 56° 48' 26" N; 132° 57' 28" W. elev. 15 m.

Voucher specimens are preserved in vitro at CLRS and JR plantations, Vancouver Island, B. C. Voucher specimens for Cedar Grove Botanical Area, John Day, OR, Mt. Angeles, WA and Mitkof Island, AK are deposited in the herbarium, Baylor University.

Fresh, frozen leaves (200 g) were steam distilled for 2 h using a circulatory Clevenger-type apparatus (Adams, 1991). The oil samples were concentrated (ether trap removed) with nitrogen and the samples stored at -20°C until analyzed. The extracted leaves were oven dried (100°C, 48 h) for determination of oil yields. Additional steam distillation tests for up to 48 h revealed that the 2 h distillation removed about 23% (of the total oil for 48 h), providing a correction factor of 4.34 (x 2h = 48 h total). The yields appeared to asymptote at about 104% of the 48 h total oil.

The oils were analyzed on a HP5971 MSD mass spectrometer, scan time 1/ sec., directly coupled to a HP 5890 gas chromatograph, using a J & W DB-5, 0.26 mm x 30 m, 0.25 micron coating thickness, fused silica capillary column (see Adams, 2007 for operating details). Identifications were made by library searches of our volatile oil library (Adams, 2007), using the HP Chemstation library search routines, coupled with retention time data of authentic reference compounds. Quantitation was by FID on an HP 5890 gas chromatograph using a J & W DB-5, 0.26 mm x 30 m, 0.25 micron coating thickness, fused silica capillary column using the HP Chemstation software. Terpenoids (as per cent total oil) were coded and compared among the species by the Gower metric (1971). Principal Coordinate Ordination (PCO) was performed by factoring the associational matrix using the formulation of Gower (1966) and Veldman (1967). Principal components analysis (PCA) follows the formulation of Veldman (1967).

Table 1. Comparison of site characteristics between JR and CLRS plantations on southern Vancouver Island, BC.

Site	Latitude	Longitude	Elevation m	MAT	MWMT	MCMT	TD	MAP	MSP
JR	48° 25' 28.0" N	124° 00' 55.9" W	105m	9.3	16	3.3	12.6	2465	364
CLRS	48° 49' 7.7" N	124° 07' 48.2" W	185m	8.9	17.2	1.9	15.3	2192	284

Site	SHM	SNOW	SI	SOILS	BEC variant	Site association
JR	44	0	32	glacial till/Podzolic sandy loam	CWhxm2	1
CLRS	61	100	20	glacial till/Podzolic gravelly sandy loam	CWhvm1	5

Table notes:

MAT -mean annual temperature; MWMT - mean temperature of the warmest month; MCMT - mean temperature of the coldest month; TD - Continentality (MWMT-MCMT); MAP - mean annual precipitation; MSP - mean summer precipitation; SHM - moisture index=(MWMT/(MSP/1000)), SNOW - mean height (m) at 50 years; SI - site index=productivity; BEC variant - xm2=dry maritime; vm1=very moist maritime; Site association - 1=zonal; 5=wetter and richer.

RESULTS AND DISCUSSION

Case I. Oils from Rennell Sound Pass trees grown in two plantations (CLRS and JR).

ANOVA of the terpenoids (as % total oil) plus total % oil yields from Rennell Sound Pass samples grown at CLRS and JR plantations revealed two significant (myrcene, α-terpinyl acetate) and

one highly significant (terpinolene) difference (Table 1). No significant difference was found in % oil yield. No significant differences were found among the major components (cf. α-pinene, δ-3-carene, limonene, β-phellandrene, Table 1). ANOVA of the terpenoids on a mg/g basis gave similar results with the same three monoterpenes being significantly different (Table 2).

To visualize the similarities using % data, a similarity matrix was assembled and factored. Six eigenroots of 24.7, 21.79, 16.6, 10.69, 9.79, and 6.52% accounted for 89.6% of the variation among the 9 samples. The large number of eigenroots (6) from such a small number of samples (9) indicates that the data set is highly variable. PCO shows the CLRS and JR samples are intermixed, not separated by plantation site (Fig. 1). An analysis using mg/g data for each terpenoid gave a similar ordination (Fig. 2).

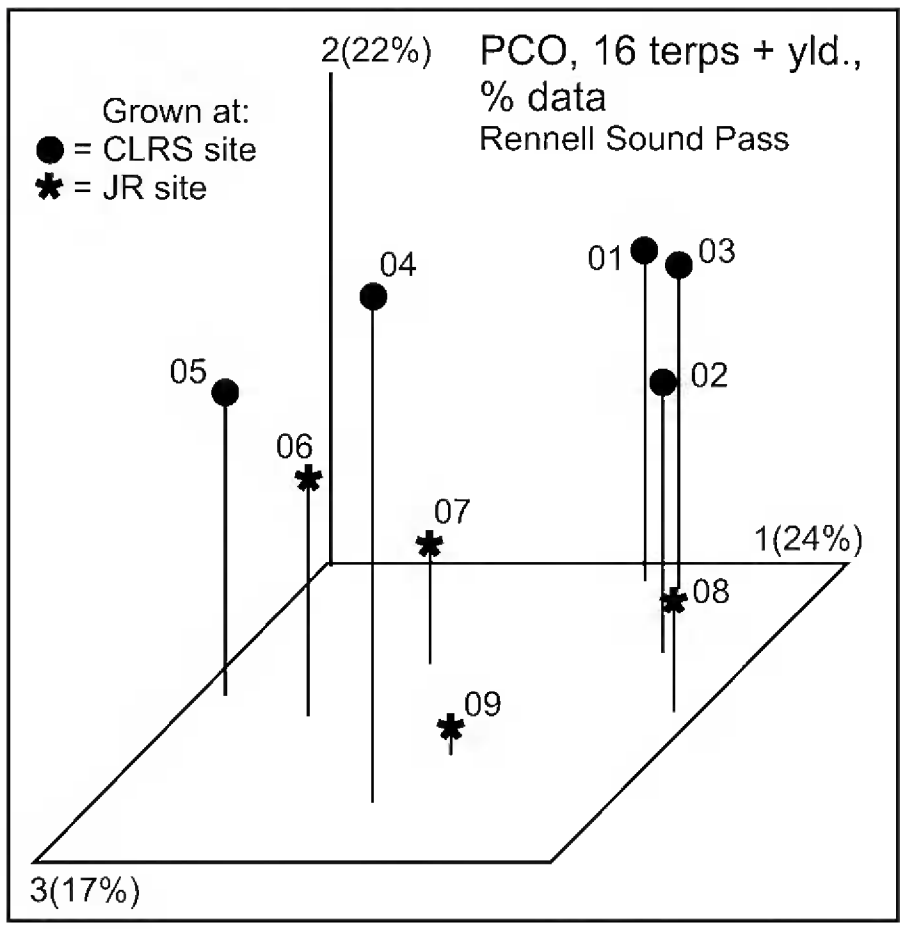

Figure 1. PCO using % total oil data, for Rennell Pass trees grown at the CLRS and JR plantations.

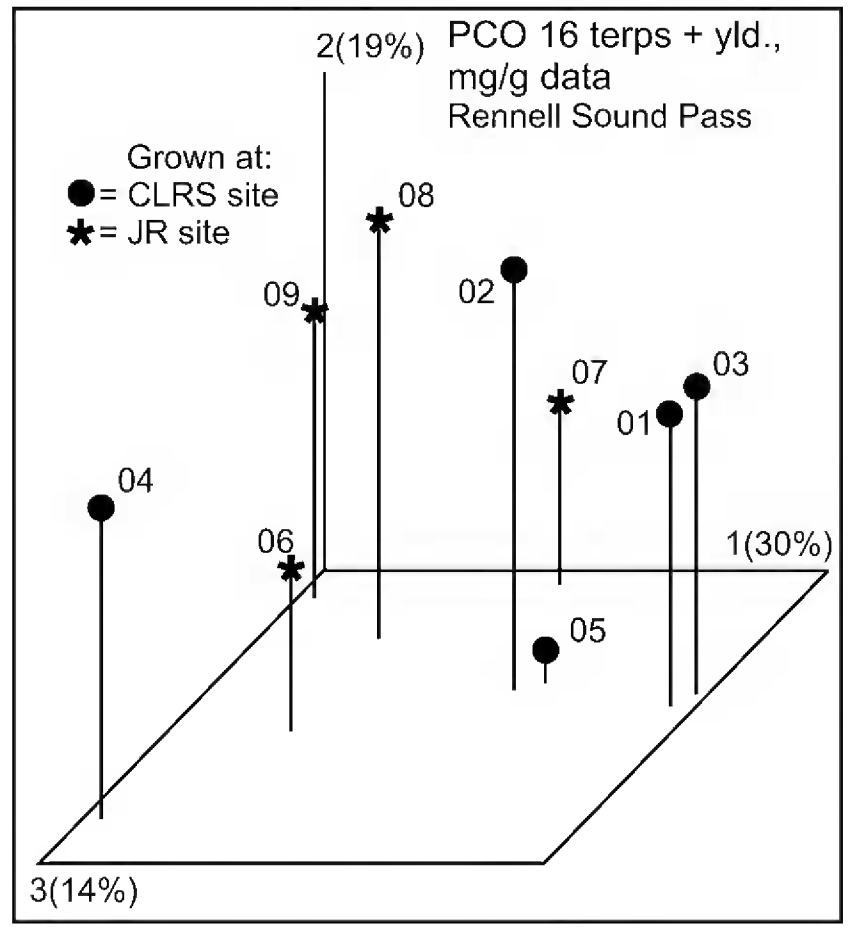

Figure 2. PCO using mg/g data, for Rennell Pass trees grown at the CLRS and JR plantations.

Examination of the oils from trees at CLRS and JR (Table 3) shows there is considerable variation within the CLRS and JR samples. It seems likely that genetic differences among trees are the major factor in the terpene levels.

To examine the correlation among individual terpenes and % oil yield, PCA was performed on the correlation matrix of the 16 terpenoids (plus % oil yield). Three eigenroots accounted for 33.9, 26.0, and 17.9% (77.7% total) of the variance among the samples. Ordination of the terpenoids shows the patterns of correlation (Fig. 3).

Percent oil yield was negatively correlated with APNN (-0.54) and BPNN (-0.54) and positively correlated with LMNN (0.54) and BPHL (0.54). Many of the monoterpenes are highly correlated forming a

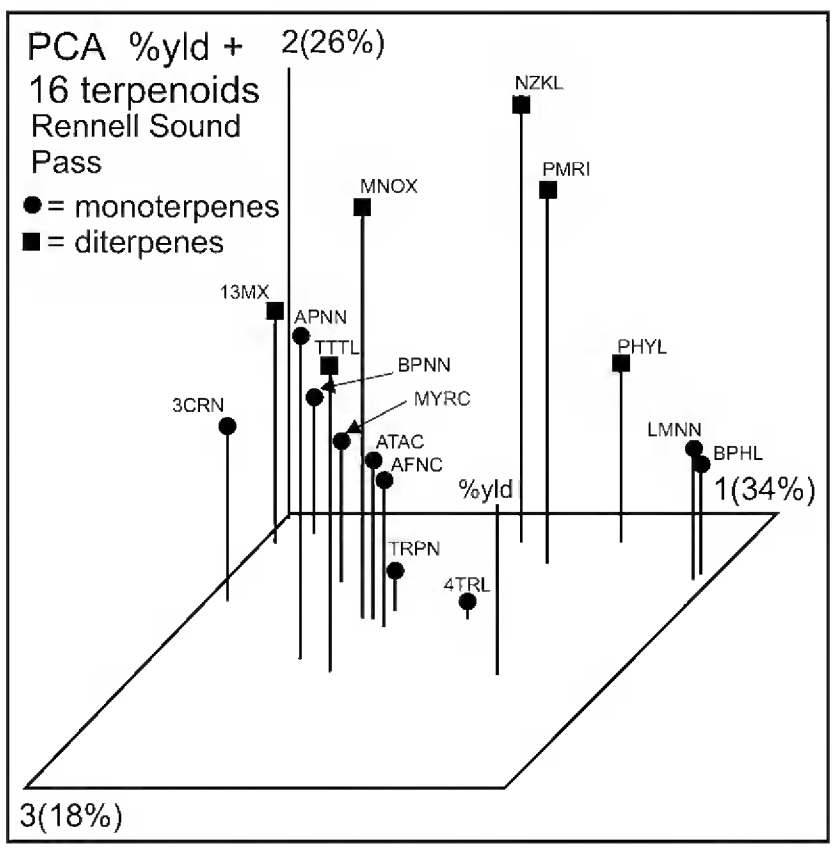

Figure 3. PCA ordination showing correlation between yield, monoterpenes and diterpenes.

group (Fig. 3, left). Limonene and β-phellandrene are highly negatively correlated with α- and β-pinene, 3-carene, myrcene (-0.86 to -0.51) and this results in their separation on the first principal component (Fig. 3.).

Interestingly, some of the diterpenes (13-epi-manool, trans-totarol, manool oxide) are correlated with the α- and β- pinene, 3-carene, myrcene group and other diterpenes (nezukol, iso-pimaradiene, and phyllocladanol) are correlated with limonene and β-phellandrene (Fig. 3).

In summary, the leaf oils of trees from seed samples collected from Runnels Sound Pass, Haida Gwaii, BC and grown at the CLRS and JR plantations showed little differences in their oils. Because the samples did not group by CLRS or JR site in PCO ordination, it appears that genetic differences played a much larger role in the determination of the terpenoids profiles than differences in the environment between the CLRS and JR sites.

Case II. Oils from naturally grown compared to cultivated trees: John Day, OR population.

Callitropsis nootkatensis has three isolated, inland populations, two in southern British Columbia, and another in eastern Oregon at the Cedar Grove Botanical Area (CGBA) near John Day, OR. The John Day population is interesting because it is very isolated and also very well defined in size. Cuttings were collected and rooted about 25 years ago and planted at the CLRS plantation on Vancouver Island. Samples of fresh foliage were collected Oct 2013 from 8 trees and steam distilled for comparison with oils from 4 trees cultivated at CLRS (collected Nov. 2012) to compare leaf oil yield and terpenoid composition.

ANOVA between John Day natural and cultivated trees (Table 4, % cpd data) revealed five of the six major monoterpenes' concentrations were significantly or highly significantly different (α-pinene, β-pinene, myrcene, limonene and β-phellandrene). Percent oil yield, although a little larger in the natural population was not significant. None of the diterpenes was significantly different.

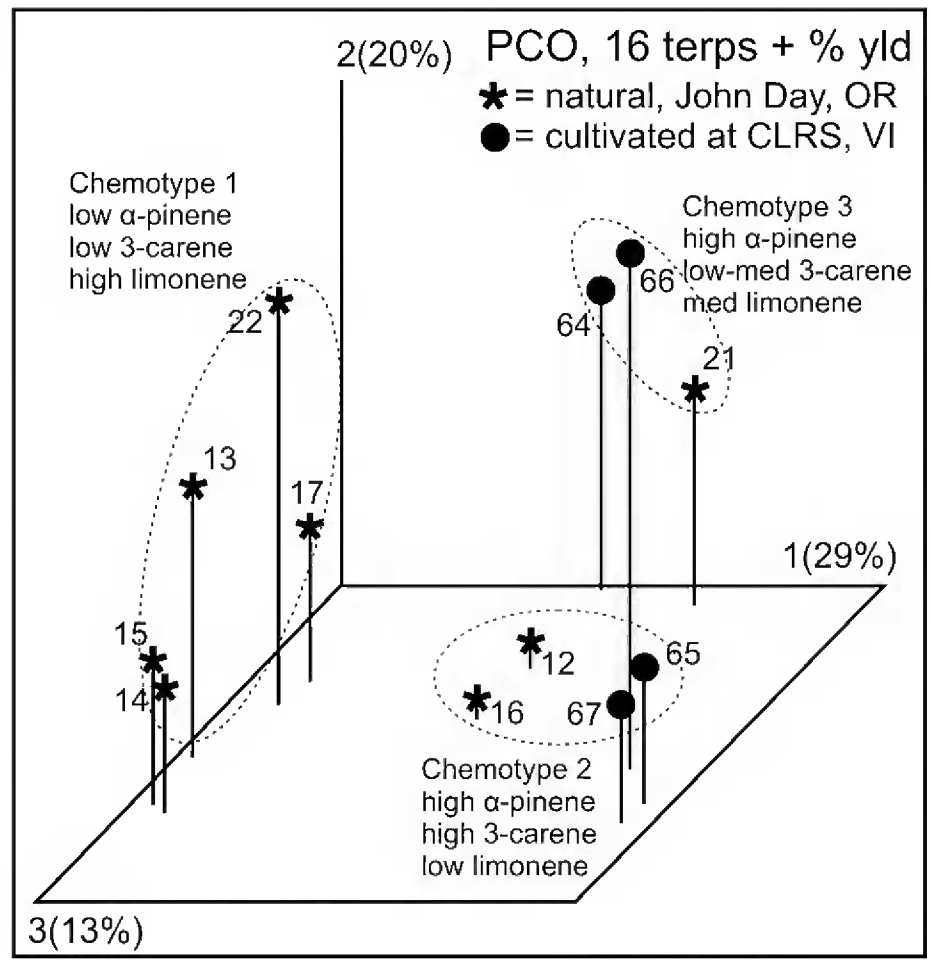

Figure 4. PCO of natural and cultivated samples (ex John Day, OR). 2 digit numbers are the last of collection number (eg., 21 = *Adams 14121*).

To visualize clustering among individuals, a similarity matrix was assembled and factored. Seven eigenroots of 29.3, 19.5, 13.5, 8.8, 7.7, 5.3 and 4.6% accounted for 88.6% of the variation among the 12 samples. The large number of eigenroots (6) from such a small number of samples (12) indicates that the data set is highly variable. PCO shows (Fig. 4) three chemotypes: 1. low α-pinene, low δ-3-carene, high limonene/ β-phellandrene; 2. high α-pinene, high δ-3-carene, low limonene/ β-phellandrene; 3. high α-pinene, low-medium δ-3-carene, medium limonene/ β-phellandrene (Fig. 4 and Table 5). CLRS samples are found intermixed with the samples from natural trees in chemotypes 2 and 3 (Fig. 4), but not in chemotype 1.

Differences in the terpenoid composition between and within chemotypes are shown in Table 5. Nezukol is highly variable within chemotypes 1 and 3, ranging from 3.3 - 9.4% and 0.2 - 6.5%, respectively. The nature of the variation suggests that these differences are not merely due to environmental factors, but likely different genotypes (chemotypes).

Correlation patterns among the 16 terpenoid concentrations and % oil yield (17 characters) were examined by PCA resulting in 3 eigenroots that accounted for 43.05, 22.14 and 17.51% of the variation among the 17 characters (82.75% of the total variance). Ordination of the 16 terpenoids plus % oil yield is shown in Figure 5. As seen in the Rennell Pass individuals (Fig. 3), LMNN and BPNN are negatively correlated with the monoterpenes. The other monoterpenes (APNN, 3CRN, BPNN, AFNC, MYRC, TRPN) are highly correlated and cluster (Fig. 5, left). Oil yield (% yld) has only low correlations (0.55 ATAC, -0.55 NZKL, 0.53 LMNN) as found in Case I (Fig. 3).

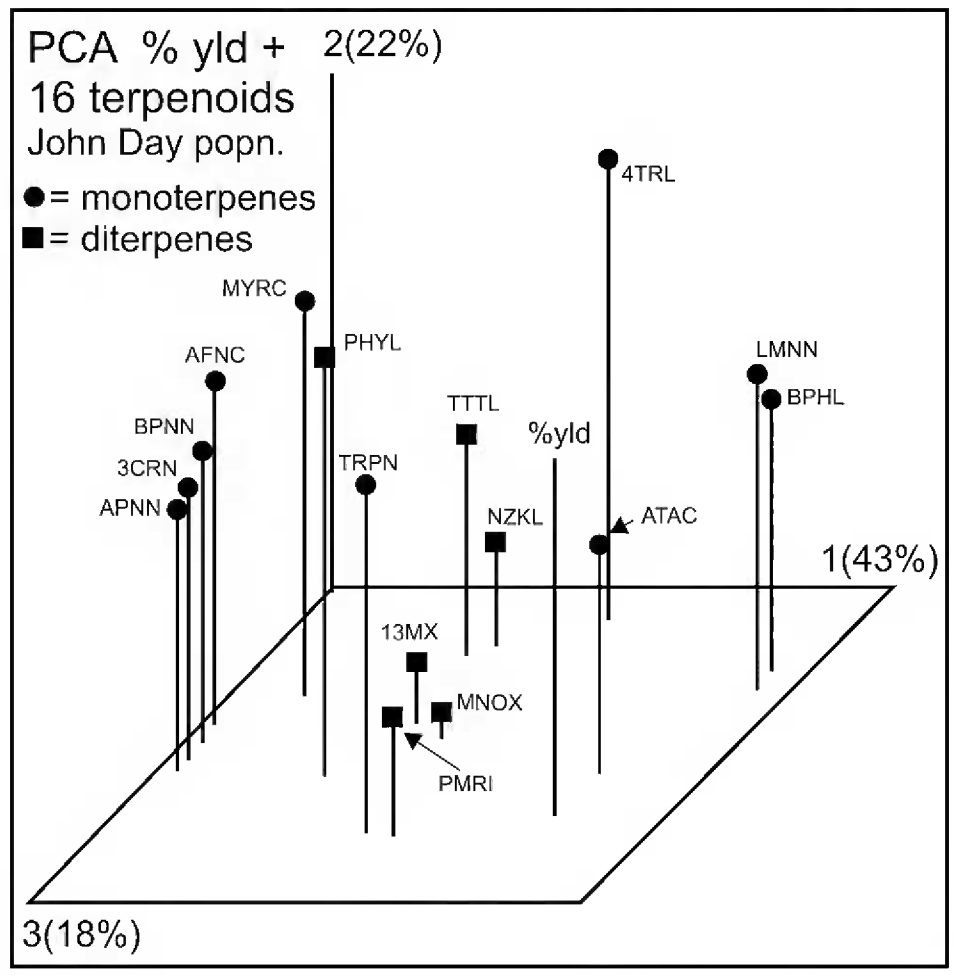

Figure 5. PCA showing the correlation patterns among terpenes and oil yield in the John Day popn.

In summary, as in Case I, the environmental contribution to sample ordination seems less than the variation due to genetic differences as evidenced by the grouping of CLRS and natural trees. The large differences among the chemotypes in the concentrations of major terpenoids (Table 5) supports genetic control of terpene synthase activities of individuals, rather than merely environmental modification of enzyme pathways.

Case III. Oils from naturally grown compared to cultivated trees: Mt. Angeles (lower slope), ONP population.

Seeds were collected from trees on Mt. Angeles, ONP, germinated and planted at the JR site in 1994, thus producing mature trees for sampling (Nov. 2012). In addition, samples were collected from trees growing naturally on the lower slopes of Mt. Angeles for terpene analysis. ANOVA between Mt. Angeles natural and cultivated samples revealed (Table 6, % total oil basis) terpinolene and nezukol were highly significantly different, and trans-totarol was significantly different. Percent oil yield, although a little smaller in the natural population, was not significant. The differences between the major compounds were usually not significant, except for nezukol (5.32% natural, 17.2% cultivated, Table 6.).

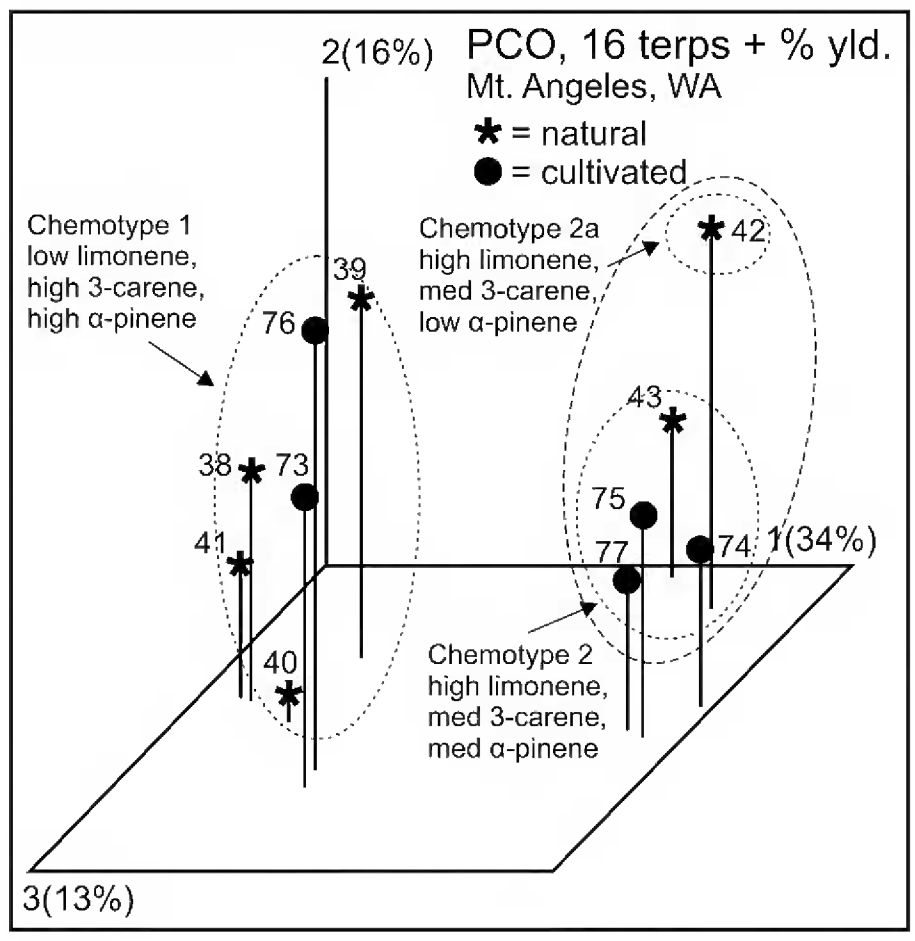

Fig. 6. PCO. Mt. Angeles, natural vs cultivated.

The similarity matrix among the samples was factored and gave five eigenroots before they began to asymptote. The five eigenroots accounted for 34.1, 15.7, 12.7, 9.9, and 6.9% of the variation among the 11 samples (79.9% of total). In the PCO of the similarity matrix, axis 1 separates the samples into two chemotypes: 1. low limonene/ β- phellandrene, and high δ-3-carene and α-pinene and 2. high limonene/ β- phellandrene, medium δ-3-carene and medium or low α-pinene (Fig. 6). Chemotype 2 can be further subdivided by having medium amounts of α-pinene compared to low α-pinene (2a, Fig. 6).

The two major chemotypes are interspersed in both the cultivated and natural samples (Fig. 6). The chemotypes seem to be little affected by the different environments at JR or Mt. Angeles, suggesting that genetics is the major factor in the maintenance of the chemotypes.

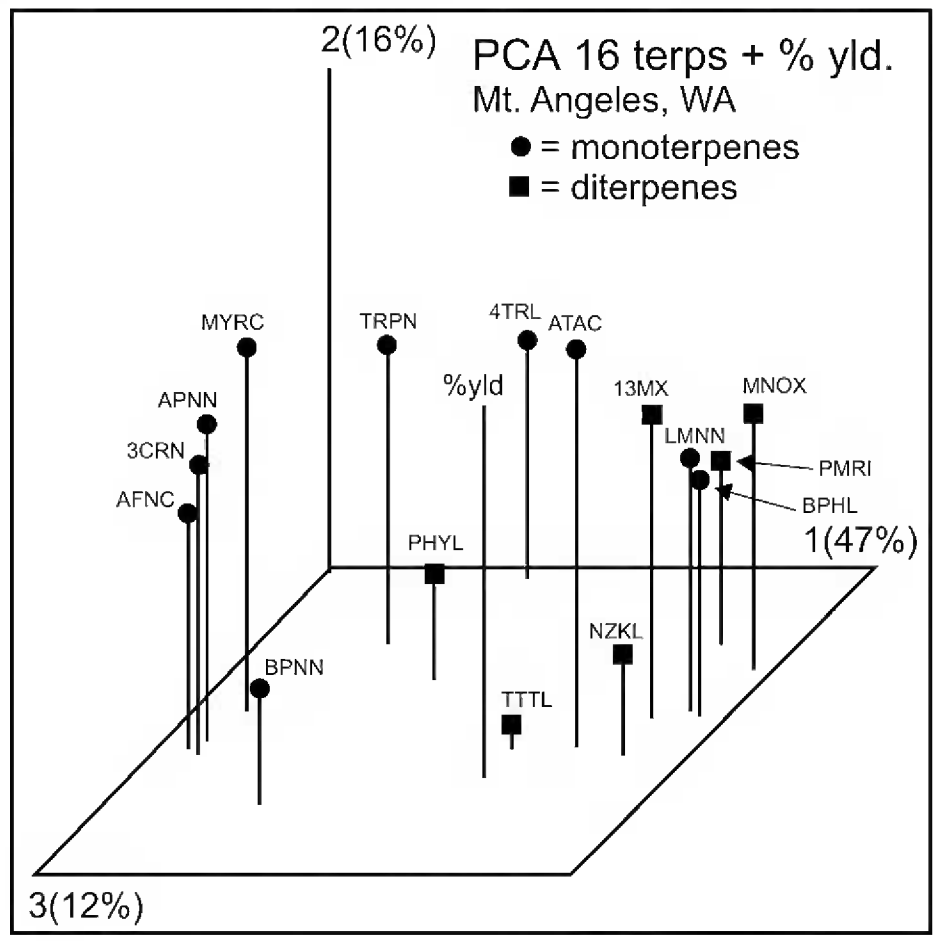

Figure 7. PCA, 16 terpenoids plus % yield, Mt. Angeles, WA.

Table 7 shows the variation among the 3 chemotypes in representative samples of both cultivated and natural origins. Although chemotype 1 is uniformly high in α-pinene and δ-3-carene, with low limonene and β-phellandrene, nezukol varies from 3.4 to 17.7%. This same polymorphism is seen in chemotype 2, where nezukol ranges from 3.9 to 17.3% (Table 7).

The patterns of correlation among the 16 terpenoids and % oil yield (17 characters) were examined by PCA, which gave four eigenroots accounting for 47.3, 16.1, 12.2 and 10.3% of the variation among the 17 characters (85.9%) of the variance). Ordination of the terpenoids plus % yield is shown in Figure 7. The pattern is similar to that found for the John Day data (Fig. 5), except the diterpene group (MNOX, 13MX, PMRI) is more closely associated with LMNN/BPHL. Once again, % oil yield is not strongly correlated (0.58 - LMNN and 0.58 - BPHL).

The major pattern found in PCO was the interspersion of samples from cultivated and natural trees in the two major chemotypes (Fig. 6). This suggests that the major factor in determining the observed ordination is genetic differences.

Case IV. Oils from naturally grown compared to cultivated trees: Mitkof Island, AK population.

Cuttings were collected from trees on Mitkof Island, AK and subsequently, rooted cuttings were planted at the CLRS site in 1988 and leaf samples were taken (Nov. 2012) from 25 year old trees. In addition, samples were collected from trees growing naturally on Mitkof Island, AK (as near to the original collection site as possible) for terpene analysis.

ANOVA between natural samples from Mitkof Island and cultivated Mitkof Island trees (Table 8) revealed no significant differences in either the % oil yield or the concentrations of any of the 16 terpenoids.

PCO of the similarity matrix among the samples and ordination shows two chemotypes (Fig. 8, chemotypes 1 and 2). Chemotype 1 contains two cultivated trees (*13713, 13714*) and a natural tree (*14153*) whose oils have large amounts of α-pinene (24.9 - 31.0%), low amounts of limonene and β-phellandrene (2.5 - 5.5%) and moderate amounts of nezukol (7.1 - 11.3%). A fourth sample (natural, *14152*) is similar, but differs in having lower α-pinene and a large amount of phyllocladanol (14.9%, Table 9, Chemotype 1a). In fact, this was the only sample (in either cultivated or natural groups) that had phyllocladanol (14.9%).

Chemotype 2 in PCO (Fig. 8) is composed of samples from 3 cultivated trees (13710, 13711, 13712) and 3 from natural trees (14149, 14150, 14151). The compositions of four of these samples are given in Table 9 (in boldface). All of these samples have low amounts of α-pinene (7.7 - 13.8%), large amounts of limonene/ β-phellandrene (14.0 - 18.9%) and moderate amounts of nezukol (5.5 - 10.8%). These data make it quite clear that there are 2 (and perhaps 3) chemotypes in the Mitkof Island population.

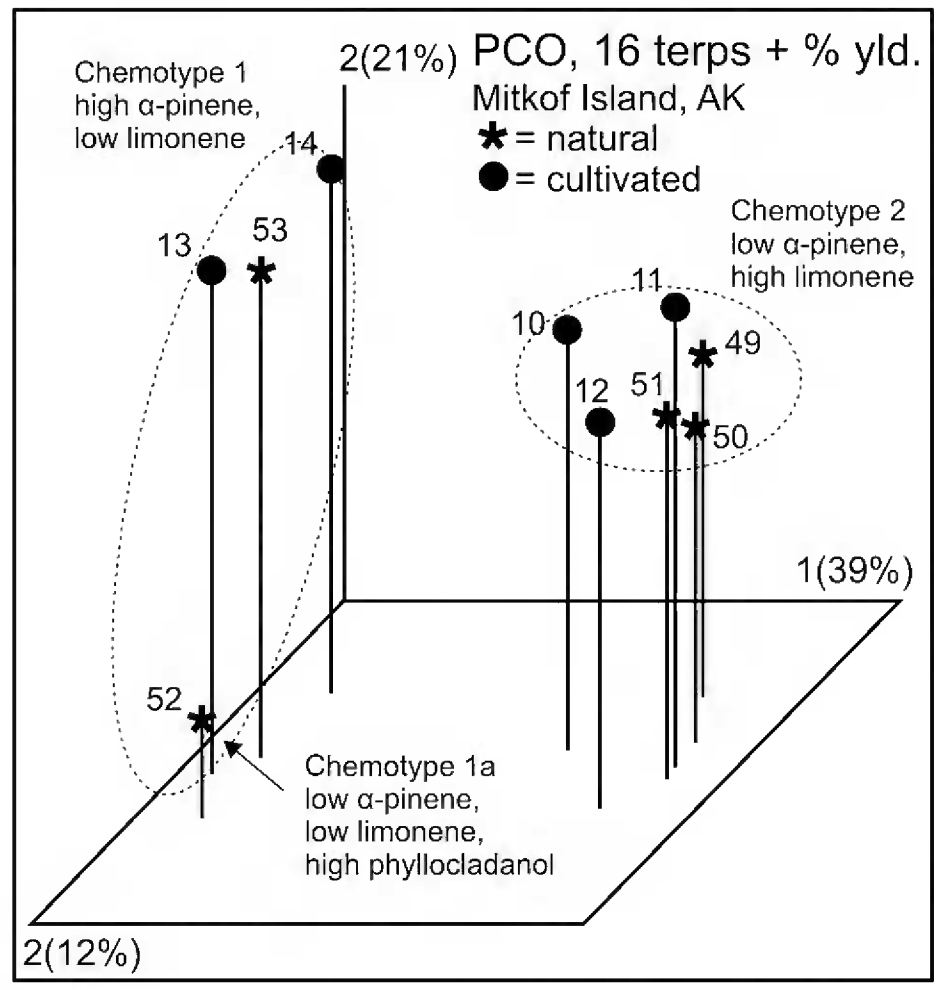

Figure 8. PCO of natural and cultivated Mitkof Island, AK samples.

The fact that five of the samples came from cultivated trees on Vancouver Island and five came from a natural population on Mitkof Island did not appear to affect chemotypes compositions. In short, these chemotypes appear to be under strong genetic control of their terpene synthases.

To examine the pattern of correlation among the terpenes, PCA was performed and factoring the correlation matrix resulted in eigenroots that accounted for 46.8, 26.5, 10.2, 6.7 and 5.1% of the variation (total 95.3%). Ordination of the terpenoids (Fig. 9) show a quite different pattern than previously seen in the other related cases. The diterpenes are strongly correlated and form a tight group. Monoterpenes are in three groups: 1. α- and β- pinene, myrcene and δ-3-carene; 2. the balance of the monoterpene hydrocarbons plus α-terpinyl acetate and oil yield; and 3. terpin-4-ol (Fig. 9).

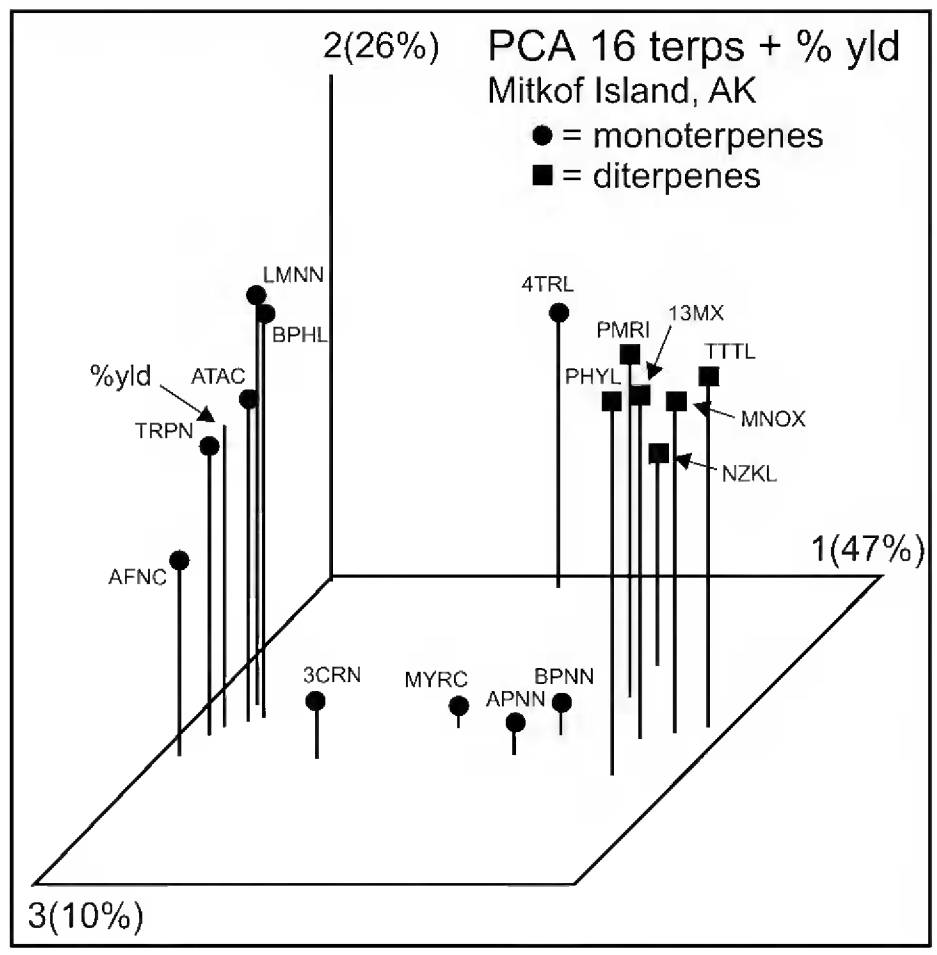

Figure 9. PCA, Mitkof Island, AK.

The highest correlations of oil yield are with α-terpinyl acetate (0.67) and terpinolene (0.61). The first axis removed 47% of the variation among the components. This may be due to the two chemotypes.

The Mitkof Island case presents the clearest evidence that differences in the oils of samples cultivated or from natural trees pale in comparison to the differences in individuals of either chemotype 1 or 2. There are surely environmentally induced differences between cultivated and natural tree oils from Mitkof Island, but they seem to be minor compared to the genotypic (chemotype) differences seen among these trees.

CONCLUSIONS

In general, the four cases examined showed similar results in that the samples from cultivated and natural trees were interspersed in PCO rather than clustering by source. This implies that genetics (chemotypes) plays a larger role than environmental factors for the cases examined. In all cases but the first, chemotypes were found and these exhibited large compositional differences from other oils in the same plot or natural population. These chemotypes (genotypes) persisted whether in cultivation or in the natural population. This is highly suggestive that genetics plays a strong role in the expression of terpene synthase genes in *C. nootkatensis*. Unexpectedly, the yields of oils from cultivated and natural trees were not significantly different in any of the four cases. However, it should be noted that all the samples were intentionally collected during the fall to minimize seasonal variation. So seasonal variation should not be dismissed as insignificant. The use of data, from either or both the CLRS, JR sites and/or from natural populations, appear to be justified by our studies.

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LITERATURE CITED

Adams, R. P. 1991. Cedarwood oil - Analysis and properties. pp. 159-173. in: Modern Methods of Plant Analysis, New Series: Oil and Waxes. H.-F. Linskens and J. F. Jackson, eds. Springler- Verlag, Berlin.

Adams, R. P. 2007. Identification of essential oil components by gas chromatography/ mass spectrometry. 4th ed. Allured Publ., Carol Stream, IL.

Adams, R. P., P. Thomas and K. Rushforth. 2007. The leaf essential oils of the new conifer genus, *Xanthocyparis*: *Xanthocyparis vietnamensis* and *X. nootkatensis*. J. Ess. Oil Res. 19: 30-33.

Cheng, Y. S. and E. von Rudloff. 1970. The volatile oil of the leaves of *Chamaecyparis nootkatensis*. Phytochemistry 9: 2517 - 2527.

Cool, L. 2001. Ent-daucane and acorane sesquiterpenes from *xCupressocyparis leylandii* foliage. Phytochemistry 58: 969-972.

Gower, J. C. 1966. Some distance properties of latent root and vector methods used in multivariate analysis. Biometrika 53: 326-338.

Gower, J. C. 1971. A general coefficient of similarity and some of its properties. Biometrics 27: 857-874.

Veldman, D. J. 1967. Fortran programming for the behavioral sciences. Holt, Rinehart and Winston Publ., NY.

von Rudloff, E. 1975. Volatile leaf oil analysis in chemosystematic studies of North American conifers. Biochem. Syst. Ecol. 2: 131-167.

Yatagai, M., T. Sato and T. Takahashi. 1985 Terpenes of leaf oils from *Cupressaceae*. Biochem. Syst. Ecol. 13: 377-385.

Table 1. ANOVA of % oil yields and leaf terpenoid concentrations (as % total oil) of *C. nootkatensis* from Rennell Pass grown at CLRS and JR plantations. ns = not significant at P≤0.05. * = significant (P≤ 0.05); ** ≤ highly significant (P ≤ 0.01). The 3 components that vary significantly between CLRS and JR are in boldface. Oil yields 2h corrected to 48h yield by correction factor of 4.34. Codes are used in the PCA figures throughout this paper.

KI	code	component	CLRS	JR	F ratio	P, signif.
	%yld	% oil yield (48h)	5.16	4.64	0.54	.508 ns
932	APNN	α-pinene	21.6	17.2	1.03	.347 ns
945	AFNC	α-fenchene	0.7	0.9	0.11	.751 ns
974	BPNN	β-pinene	2.5	2.0	1.21	.308 ns
988	**MYRC**	**myrcene**	**3.2**	**2.2**	**9.74**	**.016** *
1008	3CRN	δ-3-carene	22.8	19.1	4.00	.084 ns
1024	LMNN	limonene	11.4	14.3	0.24	.642 ns
1025	BPHL	β-phellandrene	11.4	14.4	0.23	.647 ns
1086	**TRPN**	**terpinolene**	**4.2**	**3.0**	**13.21**	**.008** **
1174	4TRL	terpinen-4-ol	0.8	0.3	3.16	.117 ns
1346	**ATAC**	**α-terpinyl acetate**	**1.1**	**0.6**	**5.92**	**.044** *
1958	PMRI	pimaradiene<iso->	0.3	0.5	0.90	.624 ns
1987	MNOX	manoyl oxide	1.3	1.6	0.22	.881 ns
2009	13MX	13-epi-manool oxide	0.4	0.3	1.00	.648 ns
2132	NZKL	nezukol	7.6	12.9	4.09	.081 ns
2209	PHYL	phyllocladanol	0.3	1.6	1.84	.216 ns
2314	TTTL	trans-totarol	0.9	0.5	0.30	.605 ns

Table 2. ANOVA of oil yields and terpenoid concentrations as mg/ g DW of *C. nootkatensis* from Rennell South Pass grown at CLRS and JR plantations. ns = not significant at P≤0.05. * = significant (P≤ 0.05); ** ≤ highly significant (P ≤ 0.01). The 3 components that vary significantly between CLRS and JR are in boldface.

KI	component	CLRS	JR	F ratio	P, signif.
	oil yield (mg/g, 48h)	51.6	46.4	0.54	.508 ns
932	α-pinene	2.40	1.80	2.54	.153 ns
945	α-fenchene	0.10	0.09	0.44	.532 ns
974	β-pinene	0.27	0.20	2.56	.151 ns
988	**myrcene**	**0.37**	**0.24**	**18.82**	**.004 ****
1008	δ-3-carene	0.27	0.20	4.34	.074 ns
1024	limonene	1.53	1.49	0.01	.951 ns
1025	β-phellandrene	1.53	1.49	0.01	.944 ns
1086	**terpinolene**	**0.56**	**0.31**	**6.78**	**.034 ***
1174	terpinen-4-ol	0.09	0.05	2.28	.173 ns
1346	**α-terpinyl acetate**	**0.14**	**0.06**	**5.53**	**.048 ***
1958	pimaradiene<iso->	0.03	0.04	0.32	.592 ns
1987	manoyl oxide	0.17	0.15	0.15	.708 ns
2009	13-epi-manool oxide	0.05	0.03	1.29	.293 ns
2132	nezukol	0.83	1.52	2.97	.126 ns
2209	phyllocladanol	0.02	0.26	1.69	.233 ns
2314	trans-totarol	0.10	0.07	0.712	.569 ns

Table 3. Variation among the leaf essential oils (as % total oil) of *C. nootkatensis* from Rennell Sound Pass grown at CLRS and JR plantations. Note: 06 = *Adams 13706*, 08 = *Adams 13708*, 09 = *Adams 13709*, 01 - *Adams 13701*, 04 = 13704, 05 = *Adams 13705*, in Figs. 1 and 2. The 3 components that vary significantly between CLRS and JR are in boldface.

KI	component	JR *13706*	*JR 13708*	*JR 13709*	CLRS *13701*	CLRS *13704*	CLRS *13705*
	% oil yield (48h)	4.81	4.64	3.82	5.90	3.12	6.25
932	α-pinene	17.6	21.8	14.0	18.1	35.4	9.3
945	α-fenchene	1.0	0.8	0.7	1.1	0.8	0.7
974	β-pinene	1.3	2.1	1.4	2.1	3.8	1.1
988	**myrcene**	**2.3**	**2.1**	**1.9**	**2.7**	**4.3**	**2.8**
1008	δ-3-carene	19.5	21.1	17.0	25.2	26.7	15.8
1024	limonene	18.5	7.5	15.4	11.9	4.7	24.6
1025	β-phellandrene	18.4	7.5	15.4	12.0	4.7	24.6
1086	**terpinolene**	**3.8**	**2.0**	**3.3**	**4.4**	**4.1**	**4.3**
1174	terpinen-4-ol	0.6	0.2	0.3	0.8	0.8	0.9
1346	**α-terpinyl acetate**	**0.4**	**0.4**	**0.3**	**1.0**	**0.7**	**1.3**
1958	pimaradiene<iso->	0.2	0.3	0.7	0.2	0.1	0.2
1987	manoyl oxide	0.8	1.9	1.6	1.6	0.9	0.9
2009	13-epi-manool oxide	0.2	0.5	0.3	0.5	0.3	0.3
2132	nezukol	5.7	23.0	18.7	8.5	3.6	3.6
2209	phyllocladanol	1.6	t	0.0	0.0	0.0	0.8
2314	trans-totarol	0.5	0.8	0.7	1.3	0.3	0.4

Table 4. ANOVA of % oil yields and terpenoid concentrations (as % total oil) in the leaf essential oils of *C. nootkatensis* from a natural stand (Cedar Grove Botanical Area, CGBA, John Day, OR) and trees from cuttings (ex CGBA) cultivated at CLRS. ns = not significant at P≤0.05. * = significant (P≤ 0.05); ** ≤ highly significant (P ≤ 0.01). Components that vary significantly are in boldface.

KI	component	CGBA natural	CGBA CLRS	F ratio	P, signif.
	% yield (48h)	4.38	3.52	1.36	.270 ns
932	**α-pinene**	**11.51**	**34.17**	**29.97**	**.005 ****
945	α-fenchene	0.93	0.70	2.06	.179 ns
974	**β-pinene**	**1.14**	**3.40**	**31.90**	**.004 ****
988	**myrcene**	**2.26**	**3.55**	**47.60**	**.001 ****
1008	δ-3-carene	19.38	19.27	0.00	.981 ns
1024	**limonene**	**18.99**	**7.25**	**8.01**	**.017 ***
1025	**β-phellandrene**	**18.99**	**7.25**	**8.01**	**.017 ***
1086	terpinolene	3.04	2.60	1.34	.273 ns
1174	terpinen-4-ol	0.11	0.16	nt	nt
1346	α-terpinyl acetate	0.73	0.62	0.08	.776 ns
1958	pimaradiene<iso->	0.69	1.20	3.01	.111 ns
1987	manoyl oxide	3.30	2.25	2.27	.164 ns
2009	13-epi-manool oxide	0.99	0.68	4.09	.068 ns
2132	nezukol	7.74	6.93	0.13	.723 ns
2209	phyllocladanol	0.50	0.93	0.50	.504 ns
2314	trans-totarol	0.27	0.25	nt	.nt ns

Table 5. Variation in % oil yields and terpenoid concentrations (as % total oil) in the leaf essential oils of *C. nootkatensis* for natural individuals from CGBA (John Day, OR) and cultivated individuals grown from cuttings (ex CGBA) at CLRS, Vancouver Island, BC. Notes: In Fig. 4: 13 = *Adams 14113*, 14 = *Adams 14114*, 16 = *Adams 14116*, 65 = *Adams 13665*, 67 = *Adams 13667*, 64 = *Adams 13664*, 66 = *Adams 13666*, 12 = *Adams 14121*. Chemotypes 1, 2 and 3 are shown in normal, italics and boldface.

		chemotype 1		*chemotype 2*			***chemotype 3***		
KI	component	**CGBA 14113**	**CGBA 14114**	*CGBA 14116*	*CLRS 13665*	*CLRS 13667*	***CLRS 13664***	***CLRS 13666***	*CGBA* ***14121***
	% yield (48h)	6.55	5.99	*4.43*	*3.39*	*3.47*	***4.38***	***2.91***	***3.82***
932	α-pinene	7.8	7.1	*15.0*	*34.8*	*32.4*	***24.6***	***44.9***	***22.2***
945	α-fenchene	0.7	0.8	*1.1*	*0.9*	*0.9*	***0.6***	***0.4***	***1.4***
974	β-pinene	0.8	0.8	*1.8*	*3.6*	*3.8*	***2.1***	***4.1***	***1.9***
988	myrcene	2.2	2.1	*2.4*	*3.7*	*3.4*	***3.1***	***4.0***	***2.4***
1008	δ-3-carene	11.7	15.6	*25.1*	*23.7*	*26.1*	***16.3***	***11.0***	***33.3***
1024	limonene	26.4	22.8	*11.7*	*3.0*	*3.3*	***14.2***	***8.5***	***7.2***
1025	β-phellandrene	26.4	22.8	*11.7*	*3.0*	*3.3*	***14.2***	***8.5***	***7.2***
1086	terpinolene	3.6	2.7	*4.0*	*2.6*	*3.2*	***2.6***	***2.0***	***3.4***
1174	terpinen-4-ol	0.1	0.1	*0.1*	*0.0*	*0.0*	***0.6***	***0.1***	***0.0***
1346	α-terpinyl acetate	0.8	1.7	*0.6*	*0.4*	*0.3*	***0.5***	***1.3***	***0.0***
1958	pimaradiene<iso->	0.5	0.9	*1.1*	*1.5*	*1.2*	***0.2***	***1.9***	***0.7***
1987	manoyl oxide	2.4	3.9	*4.4*	*2.7*	*3.5*	***2.4***	***0.4***	***2.8***
2009	13-epi-manool oxide	0.7	1.1	*1.1*	*0.4*	*0.8*	***9.9***	***0.6***	***1.0***
2132	nezukol	3.3	9.4	*9.7*	*11.2*	*9.8*	***6.5***	***0.2***	***5.3***
2209	phyllocladanol	0.0	0.0	*0.0*	*0.0*	*0.0*	***1.5***	***2.2***	***2.1***
2314	trans-totarol	0.1	0.2	*0.4*	*0.2*	*0.4*	***0.2***	***0.2***	***0.2***

Table 6. ANOVA of oil yields and terpenoid concentrations (as % total oil) in the leaf essential oils of *C. nootkatensis* from a natural stand (Mt. Angeles, ONP) and trees (cuttings from Mt. Angeles) cultivated at JR. ns = not significant at P=0.05. * = significant (P≤ 0.05); ** ≤ highly significant (P ≤ 0.01). Components that vary significantly are in boldface.

KI	component	Mt. Angeles natural	Mt. Angeles CLRS	F ratio	P, signif.
	% yield (48h)	4.69	4.77	0.10	.919 ns
932	α-pinene	21.72	14.70	1.73	.219 ns
945	α-fenchene	1.05	0.88	2.34	.158 ns
974	β-pinene	2.22	3.10	0.70	.571 ns
988	myrcene	2.67	2.22	4.70	.056 ns
1008	δ-3-carene	27.10	19.58	4.00	.074 ns
1024	limonene	9.15	11.92	0.46	.521 ns
1025	β-phellandrene	9.13	11.90	0.46	.522 ns
1086	**terpinolene**	**3.67**	**2.96**	**17.38**	**.003 ****
1174	terpinen-4-ol	0.78	0.48	1.51	.249 ns
1346	α-terpinyl acetate	0.38	0.86	1.52	.248 ns
1958	pimaradiene<iso->	0.32	0.48	2.56	.142 ns
1987	manoyl oxide	1.20	1.58	1.39	.268 ns
2009	13-epi-manool oxide	0.32	0.34	0.08	.775 ns
2132	**nezukol**	**5.32**	**17.20**	**50.43**	**.0002 ****
2209	phyllocladanol	1.62	0.68	1.23	.230 ns
2314	**trans-totarol**	**0.35**	**0.78**	**6.11**	**.034 ***

Table 7. Variation in oil yields and terpenoid concentrations (as % total oil) in the leaf essential oils of *C. nootkatensis* for natural individuals at Mt. Angels (Mt Ang), ONP and cultivated individuals grown from seed at JR, Vancouver Island, BC. Notes: In Fig. 6: 38 = *Adams 14138*, 40 = *Adams 14140*, 42 = *Adams 14142*, 73 = *Adams 13673*, 76 = *Adams 13676*, 43 = *Adams 14143*, 74 = *Adams 13674*, 77 = *Adams 13677*, 42 = *Adams 14142*. Chemotypes 1, 2 and 2a are in normal, boldface and boldface/italics fonts.

		chemotype 1				**chemotype 2**			***ch. 2a***
KI	component	Mt Ang 14138	Mt Ang 14140	CLRS 13673	CLRS 13676	**Mt Ang 14143**	**CLRS 13674**	**CLRS 13677**	***Mt Ang 14142***
	% yield (48h)	4.25	5.21	3.82	4.90	**7.25**	**5.47**	**4.82**	***4.04***
932	α-pinene	28.8	28.0	20.8	17.0	**13.4**	**10.6**	**10.5**	***5.3***
945	α-fenchene	1.1	1.3	1.1	1.1	**1.0**	**0.7**	**0.7**	***0.8***
974	β-pinene	2.7	2.8	2.9	7.2	**1.3**	**1.2**	**1.9**	***0.8***
988	myrcene	2.9	2.9	2.4	2.1	**2.5**	**2.0**	**2.2**	***1.9***
1008	δ-3-carene	32.5	36.6	25.7	25.8	**22.1**	**16.3**	**15.3**	***18.3***
1024	limonene	3.5	3.3	5.3	8.3	**19.9**	**16.8**	**13.8**	***18.7***
1025	β-phellandrene	3.5	3.3	5.3	8.3	**19.9**	**16.8**	**13.8**	***18.7***
1086	terpinolene	3.7	4.0	3.3	3.1	**3.6**	**2.7**	**3.1**	***3.8***
1174	terpinen-4-ol	1.2	0.4	0.6	0.1	**0.5**	**0.2**	**1.3**	***0.7***
1346	α-terpinyl acetate	0.0	1.3	0.2	0.0	**0.8**	**1.6**	**1.6**	***0.0***
1958	pimaradiene<iso->	0.4	0.2	0.4	0.3	**0.3**	**0.5**	**0.8**	***0.5***
1987	manoyl oxide	0.6	1.1	1.2	0.9	**1.4**	**1.7**	**2.1**	***2.1***
2009	13-epi-manool oxide	0.1	0.3	0.3	0.2	**0.5**	**0.4**	**0.3**	***0.4***
2132	nezukol	3.4	3.9	17.7	15.3	**3.9**	**17.3**	**18.2**	***11.5***
2209	phyllocladanol	1.4	0.0	0.1	3.3	**1.1**	**0.0**	**0.0**	***2.8***
2314	trans-totarol	0.3	0.1	1.3	0.9	**0.3**	**0.4**	**0.5**	***0.7***

Table 8. ANOVA of % oil yields and terpenoid concentrations (as % total oil) in the leaf essential oils of *C. nootkatensis* from a natural stand (Mitkof Island, AK) and trees (seeds from Mitkof Isl.) cultivated at CLRS. ns = not significant at P=0.05.

KI	component	Mitkof Island natural	Mitkof Island CLRS	F ratio	P, signif.
	% yield (48h)	3.08	2.69	1.14	.319 ns
932	α-pinene	13.34	18.38	0.69	.567 ns
945	α-fenchene	0.76	0.82	0.54	.501 ns
974	β-pinene	1.42	2.26	1.31	.286 ns
988	myrcene	2.48	2.96	2.30	.165 ns
1008	δ-3-carene	19.08	21.42	1.12	.323 ns
1024	limonene	10.32	12.74	0.25	.630 ns
1025	β-phellandrene	10.26	12.64	0.25	.635 ns
1086	terpinolene	3.94	4.06	0.41	.544 ns
1174	terpinen-4-ol	1.08	1.18	0.13	.726 ns
1346	α-terpinyl acetate	1.54	0.72	3.23	.108 ns
1958	pimaradiene<iso->	0.28	0.74	3.07	.115 ns
1987	manoyl oxide	3.14	3.02	0.02	.892 ns
2009	13-epi-manool oxide	1.14	0.90	0.37	.566 ns
2132	nezukol	11.02	7.62	3.22	.108 ns
2209	phyllocladanol	2.98	0.00	1.00	.652 ns
2314	trans-totarol	0.50	0.36	0.40	.552 ns

Table 9. Variation in oil yields and terpenoids (as % total oil) in the leaf essential oils of *C. nootkatensis* from a natural stand (Mitkof Island, AK) and trees (seeds from Mitkof Isl.) cultivated at CLRS. No significant differences were found in ANOVA. Note: In Fig. 8: 53 = *Adams 14153*, 13 = *Adams 13713*, 14 = *Adams 13714*, 52 = *Adams 14152*, 49 = *Adams 14149*, 50 = *Adams 14150*, 10 = *Adams 13710*, 11 = *Adams 13711*. Chemotypes 1, 1a and 2 (see Fig. 8) are in normal, italics and boldface fonts.

		chemotype 1			*ch. 1a*	**chemotype 2**			
KI	component	Mitkof 14153	CLRS 13713	CLRS 13714	*Mitkof 14152*	**Mitkof 14149**	Mitkof 14150	**CLRS 13710**	**CLRS 13711**
	% yield (48h)	2.53	2.47	2.39	*2.26**	**3.12**	**3.43**	**3.43**	**2.47**
932	α-pinene	31.0	31.0	24.9	*9.1*	**10.7**	**7.7**	**13.8**	**10.4**
945	α-fenchene	0.7	0.9	0.7	*0.5*	**0.8**	**0.9**	**0.8**	**0.8**
974	β-pinene	3.0	3.8	3.7	*1.3*	**1.1**	**0.8**	**1.3**	**1.5**
988	myrcene	3.2	3.4	3.6	*1.9*	**2.4**	**2.4**	**2.9**	**2.4**
1008	δ-3-carene	19.0	28.7	20.1	*14.7*	**20.5**	**20.1**	**17.6**	**20.0**
1024	limonene	3.6	2.6	5.5	*2.4*	**14.0**	**15.8**	**18.9**	**15.2**
1025	β-phellandrene	3.6	2.5	5.4	*2.4*	**14.0**	**15.7**	**18.8**	**15.1**
1086	terpinolene	2.9	4.1	3.8	*3.2*	**4.3**	**4.4**	**4.1**	**3.9**
1174	terpinen-4-ol	0.9	0.7	1.6	*0.8*	**1.7**	**1.0**	**1.3**	**1.7**
1346	α-terpinyl acetate	0.6	0.5	0.4	*0.5*	**2.2**	**2.1**	**0.3**	**1.0**
1958	pimaradiene <iso->	0.4	0.6	0.7	*0.5*	**0.3**	**0.1**	**0.4**	**1.7**
1987	manoyl oxide	2.9	3.2	3.3	*6.6*	**2.0**	**2.2**	**3.2**	**3.0**
2009	13-epi-manool oxide	0.9	1.0	0.9	*2.7*	**0.7**	**0.7**	**1.0**	**0.7**
2132	nezukol	11.3	7.1	11.3	*15.3*	**9.2**	**9.9**	**5.5**	**10.8**
2209	phyllocladanol	0.0	0.0	0.0	*14.9*	**0.0**	**0.0**	**0.0**	**0.0**
2314	trans-totarol	0.5	0.3	0.6	*1.3*	**0.3**	**0.2**	**0.2**	**0.5**

* *14152* had copious amounts of white, crystalline distillate (diterpenes) that precipitated in the condenser. Thus, the oil yield for *14152* is under estimated.